REMARKS/ARGUMENTS

Applicants note with appreciation that the Office Action dated June 2, 2004 withdrew certain objections and rejections for indefiniteness.

By this Amendment, the specification is amended to update the status of prior applications; and a spelling error is corrected. Further, claims 1, 3, 5, 6, 7, 9, 10, 13, 16 and 24 are amended. Claims 2, 4, 8, and 19-23 are cancelled. Claims 47-50 are added. An RCE is filed herewith in order to ensure entry of the present amendment, which is subsequent to a final rejection. Claims 1, 3, 5, 6, 7, 9-18, 24, 28-33 and 47-50 remain under examination.

For the convenience of the Examiner, the rejections of the present Final Office Action dated June 2, 2004, are repeated in the order set forth in that paper.

Rejections Maintained

1. Rejections of Claims 24-33 and 34-36 under 35 USC 112 as non-enabled

Rejection

The specification was said to be enabling for stimulating an immune response against a pathogen, but not for stimulating a "protective immune response that can prevent or treat infection."

Response

Applicants have shown a protective immunity in mouse experiments. However, at the time, without acquiescing in the propriety of this rejection, none of the pending claims have this recitation.

Rejection

The term "agent" was said to be not enabled in that Applicants disclose no anti-sense RNA that binds to an upstream control region and no specific antisense molecules, chelators, or amino acid sequences. (Office Action [OA], para. 13). Further, none of the claims require immunization with a Dam- bacteria (OA, para. 15).

Claims 25-33 were said to further limit independent claim 24 through requiring specific phenotypic characteristics be induced; but the agent is not claimed (OA, para. 16). Furthermore, it is asserted that claims 24-33 do not recite the step of "administering a bacteria with a mutation that alters DNA adenine methylase activity and reduces a symptom, nor do the claims recite S. typhimurium...." (OA, para. 18). The degree of alteration of *dam* is also assertedly not claimed. (OA, para. 18). It was also pointed out that the recited agent could be a polypeptide (OA, para. 20). . The enablement rejection was further based on the recited "alteration not being limited to being an alteration of the coding sequence for Dam activity in a pathogenic bacteria, the bacteria being a wild type bacteria that has already infected a subject...." (OA, para. 22). Further, "no specific genes have been defined ...outside the coding sequence of Dam activity...." (OA, para. 22).

Response

Without acquiescing in the propriety of this rejection, Applicants have amended the claims to more clearly define the "agent" claimed. For example, in claim 1, the recited agent is not one that just alters "methytransferase (Dam) activity," but, rather "an agent that prevents the bacteria's *dam* gene expression." New claim 47 is even more specific, stating that the agent is a polynucleotide. Similarly, in independent claim 24, the agent comprises a "bacteria having therein an alteration in its *dam* gene..." Independent claim 47 recites an active agent comprising "a bacteria having therein an alteration in its native *dam* gene that alters the bacteria's native level of DNA methyltransferase activity."

Rejection

Further, a screening assay for an agent was said not to describe the agent.

Response

Without acquiescing in the propriety of this rejection, Applicants are no longer presenting claims wherein the agent is defined by the results of a screening assay. It is submitted that the present claims identify distinct, particular, and enabled agents that act upon, or modify, the *dam* gene.

2. Rejections of Claims 1-18, 20-23 as indefinite

Rejection

The rejections in paragraphs 9, 10 and 12 of the previous Office Action were maintained.

Response

With regard to paragraph 9, it is believed that any contradictory claim limitations have been eliminated. With regard to both an increase and a decrease in *dam* gene expression both causing reduced virulence, this is in fact true, and is explained and documented in the specification and related *Science* article (Heithoff et al. 1999, cited by the Examiner.)

With regard to paragraph 10, the step of determining the native level of DNA methylase activity is not essential to the preparation of the recited compositions (claim1) or the method of treatment (claims 24, 32 and 47). As documented in the specification and related *Science* article (Heithoff et al. 1999, cited by the Examiner), Applicants were able to prepare such compositions and administer such treatments without such a step.

With regard to paragraph 12, the allegedly indefinite references to "increasing" or

decreasing" have been eliminated. The terms are only used in contexts where the corresponding structure is clear. That is, the terms are only used in connection with the degree of methylation of the DNA in a cell, which is a definite property.

Rejection

"It is the position of the Examiner that the claims 1-7 do not require that the resultant alterations produce strains that overproduce Dam activity, nor to be Dam- strains of bacteria; any alteration no matter how big or small a change is claimed." (OA, para. 24) An additional step of determining the native level of DNA methyltransferase activity was said to be necessary because no point of comparison is provided in the claims; "how an alteration can be determined to have been caused by the administered agent is not clearly or distinctly claimed." (OA, para. 24). The examiner further cited US 6,632,430 as teaching increased virulence with increased production of Dam, rather than attenuation. (OA. para. 25).

Response

The claims as amended do not cover any alteration that affects Dam methylase enzymatic activity. The specification describes, and the claims recite, that the alteration must reduce virulence. Furthermore, claim 1, for example, recites "an agent that prevents the bacteria's *dam* gene expression." Specific examples of such agents are particularly described in the specification. For the downward mutation, it is taught that the Dam activity should be essentially null. For the upward mutation, constitutive overproduction is shown to be sufficient. It would be a matter of routine experimentation, given the present teachings, to determine if a specific agent had sufficient impact to reduce virulence. The mechanisms of action of various polynucleotide constructs is well understood. It would be a matter of routine experimentation to determine the extent of alteration caused by the use of such agents.

With regard to US 6,632,430, the patent simply cites the present inventors' work. See Col. 1, l. 21. The reference teaches the use of inhibitors of S-adenosyl-L-methionine (SAM) dependent transmethylation or S-adenosyl homocysteine (SAH) as antimicrobials. The suggestion to also use *dam* as a target appears to be completely based on Applicants' work, and is not inconsistent therewith.

3. Rejection of Claims 1-4, 6, 8-9, 11-18, 20-21, 23-27,and 29-46 under 35 USC 102(e) over Vermeulen et al. U.S. 5,872,104

This rejection was maintained for reasons of record in paragraph 15 of the previous Office Action. It was the position of the Examiner that the teaching of Vermeulen regarding the use of sinefungin is relevant to the rejected claims in that they do not require that the agent or composition come in contact with a human or mammalian methyltransferase, only bacteria. Furthermore, it was stated, "Vermeulen et al. disclose a number of agents that will alter Dam activity, but need not directly act upon the DNA methylase protein or coding region, or upstream DNA control region, and would function to alter the bacteria's native level of DNA methylase activity." It was the position of the Examiner that claims 1 and 19 only require a change of phenotype.

Response

Vermeulen et al. 5,872,104 discloses the use of an inhibitor of RNA methylation in order to reduce the resistance of a bacterium to an antibiotic. The reference contains no mention of altering Dam methylase activity. The patent states:

"It is proposed that dam methylation might lead to more efficient transcription at promoters near oriC which may stimulate initiation of DNA replication at oriC. Recent studies also reportedly showed reduced frequency of transformation of oriC plasmids in dam mutants, and poor functioning of oriC plasmids derived from a dam mutant in an in vitro DNA replication system. This leads the inventors to predict that methylation inhibitors will likely slow down the efficiency transcription and replication of plasmids."

However, no specific inhibitors of Dam methylase are disclosed. Thus, the

reference cannot anticipate or render obvious claims 1-4, 6, 8-9, 11-18, 20-21, 23-27, and 29-46.

4. Rejection of Claims 1-5, 7,10,19 and 22 under 35 USC 102(b) over Blyn eta l.

This rejection was maintained for reasons of record in paragraph 16 of the previous Office Action. The bacterial strains of Blyn et al. were said to have been contacted with an agent that altered the bacteria's native level of DNA methyltransferase activity. The transformed E. coli reportedly expressed aberrantly high levels of Dam.

Response

Blyn et al. describe the regulation of the *pap* pilin phase variation in E. coli. The authors found that the *papA* gene transcription was affected by Dam methylation at two sites: $GATC_{1028}$ and $GATC_{1130}$. Dam methylase levels also affected the level of *pap* transcription. *dam*- E. coli strains were constructed for these studies. These constructs are not described in terms of their genotype (see p. 4053, Col. 2, "Construction of congenic *dam*- E. coli strains.") They were used to determine that mutations in the *dam* gene affected transcript initiation at the *papBA* promoter. There is no suggestion or teaching in the reference that Dam methylase levels are related to attenuation or immunogenicity of pathogenic bacteria. In a brief introductory paragraph, the authors suggest that reduction of *pap* expression may result in diminished immunogenicity. This is contrary to the operation of the present methods. Applicants have found that inhibition of the *dam* gene increases immunogenicity of the affected bacteria.

Thus Blyn et al. neither anticipates nor renders obvious claims 1-5, 7,10,19 and 22.

Conclusion

It is believed that the present Amendments place the application in condition for

allowance. Entry of this amendment pursuant to the RCE and allowance of claims Claims 1, 3, 5, 6, 7, 9-18, 24, 28-33 and 47-50 is respectfully requested. Such action, as well as the timely issuance of a Notice of Allowance is earnestly solicited. If a telephone conference would be useful in this case, the Examiner is encouraged to call the undersigned at the number below to discuss any prosecution issues.

Respectfully submitted,

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